REMARKS

Applicants' representatives wish to thank Examiner Nickol for meeting on June 24, 2003, and for his many helpful suggestions and comments.

Substance of the interview

At the interview, the pending claims were discussed and options for overcoming the 35 USC § 112 rejections. The Examiner's suggestions have been incorporated into the amendments.

Amendments

Support for the amendments can be found in original claims 24-30, 32, 60 and 67. No new matter has been added. Claims 24-35, 60-62 and 67 are now present and pending in the application.

REQUEST FOR RECONSIDERATION

Angiogenesis--the growth of new vessels from pre-existing vessels--is a complex phenomenon that occurs in distinct phases and relies upon modulation or expression of a variety of intracellular proteins, extracellular matrix components, proteases, inflammatory molecules, chemokines, molecules involved in cell division and proliferation, cytoskeletal rearrangement, adhesion and apoptosis. These new vessels serve not only to supply normal growing tissues with a vital blood supply, but can also be hoodwinked into supplying tumors. One tactic to combat tumors is to prevent angiogenesis from occurring or halt its progress in tumors.

Rejections under 35 USC § 112, first paragraph

The rejections of the claims under 35 USC § 112 are respectfully traversed. The specification teaches one of skill in the art how to use PRO-C-MG.2 (SEQ ID NO:2) to inhibit or promote angiogenesis, especially in light of PRO-C-MG.2's structural motifs, sequence homologies and expression pattern, as well as the state of the art at the time of filing.

The requirements of 35 USC §112, first paragraph require nothing more than objective enablement (*In re Marzocchi*, 439 F.2d 220). To maintain its rejection, "it is encumbent upon

DECLARATION

Applicants thank the Office for pointing out the defective Declarations. New Declarations in compliance with 37 CFR §1.67(a) identifying this and the provisional application by application numbers and filing dates are herewith provided.

IN THE SPECIFICATION

Please amend the paragraph at page 37, lines 4-11 (of the specification as originally filed) as follows:

Unless specifically stated otherwise, all % amino acid sequence identity values used herein are obtained as described above using the ALIGN-2 sequence comparison computer program. However, % amino acid sequence identity can also be determined using the sequence comparison program NCBI-BLAST2 (Altschul et al., Nucleic Acids Res. 25:3389-3402 (1997)). ~~The NCBI-BLAST2 sequence comparison program can be downloaded from http://www.ncbi.nlm.nih.gov~~. NCBI-BLAST2 uses several search parameters, wherein all of those search parameters are set to default values including, for example, unmask = yes, strand = all, expected occurrences = 10, minimum low complexity length = 15/5, multi-pass e-value = 0.01, constant for multi-pass = 25, dropoff for final gapped alignment = 25 and scoring matrix = BLOSUM62.

Please amend the paragraph at page 39, lines 21-28 (of the specification as originally filed) as follows:

Unless specifically stated otherwise, all % nucleic acid sequence identity values used herein are obtained as described above using the ALIGN-2 sequence comparison computer program. However, % nucleic acid sequence identity can also be determined using the sequence comparison program NCBI-BLAST2 (Altschul et al., Nucleic Acids Res. 25:3389-3402 (1997)). ~~The NCBI-BLAST2 sequence comparison program can be downloaded from http://www.ncbi.nlm.nih.gov.~~ NCBI-BLAST2 uses several search parameters, wherein all of those search parameters are set to default values including, for example, unmask = yes, strand = all, expected occurrences = 10, minimum low complexity length = 15/5, multi-pass e-value = 0.01, constant for multi-pass = 25, dropoff for final gapped alignment = 25 and scoring matrix = BLOSUM62.

the Patent Office [. . .]to explain why it doubts the truth or accuracy of any statement in a supporting disclosure *and to back up* assertions of its own with *acceptable evidence or reasoning*. . . " (*Marzocchi,* at 224; emphasis added). The Office has not met its burden to maintain the rejection: reasoning that simply casts doubt is not convincing evidence or reasoning.

Because of its up-regulation in angiogenesis and its likely phosphorylation activity, one of skill in the art would know how to use PRO-C-MG.2 to modulate the process of new blood vessel formation. For example, application of a kinase inhibitor would inactivate any phosphorylation activity of PRO-C-MG.2; likewise, kinase activators would promote angiogenesis by stimulating any PRO-C-MG.2 phosphorylation activity. Such inhibitors, for example, could be administered to sites of tumorigenesis to halt the growth of the tumor by interfering with vascularization, a prerequisite for tumor growth.

These conclusions could be reached by one of skill in the art because of the teachings of the specification as well as the state of the art at the time of filing. The specification teaches, and analysis of the PRO-C-MG.2 polypeptide primary sequence leads to the conclusion, that the protein has the following structural and functional features:

(1) a PX kinase domain

(2) 1-2 sites for casein II kinase phosphorylation

(3) a protein kinase domain

(4) cAMP/GMP-dependent phosphorylation sites

(5) a coiled-coil domain

(6) nuclear localization

(7) no signal sequence

Given the phosphorylation sites and definitive kinase domain, PRO-C-MG.2 is clearly involved in intracellular signaling. Adding the characteristics of a coiled-coil domain and nuclear localization, it is almost certain that PRO-C-MG.2 is important in gene expression, either as a transcription factor, part of higher-order chromatin structure that affects gene expression, or other house-keeping-like function, such as RNA transport. Finally, because the expression of PRO-C-MG.2 is up-regulated four-fold in an *in vitro* model of angiogenesis, the polypeptide is less likely to be a component of chromatin or part of the house-keeping machinery; but rather, directly involved in expression, such as a transcription factor or a kinase that phosphorylates

polypeptides that influence angiogenic-specific gene expression. Regardless of the identity of the molecule, how to make and use are the same.

The specification teaches the distinguishing characteristics of the polypeptide of the invention, PRO-C-MG.2, as discerned from the primary structure (page 128, lines 19-28). These characteristics are summarized below in Table 1.

Table 1 Distinguishing characteristics of PRO-C-MG.2 as evidenced by primary structure

Feature	# of domains	Residues	Function
cAMP/gAMP dependent protein kinase phosphorylation site	3	54-58 441-445 464-468	Phosphorylation target
Casein kinase II phosphorylation site	10	32-36 57-61 110-114 179-183 190-194 216-220 233-237 402-406 452-456 470-474	Phosphorylation target
Tyrosine kinase phosphorylation site	2 (overlapping)	116-125 117-125	Phosphorylation target
N-myristoylation sites	3 (2 overlap)	489-495 545-551 549-555	Sites of myristoylation modification, which insert into plasma membrane, anchoring the polypeptide to the membrane.
Leucine zipper	1	289-316	Can bind DNA, dimerize with other polypeptides
PX kinase domain	1	16-122	Phosphorylation activity; some PX

			domains bind phosphoinositides
pkinase (protein kinase) domain	1	230-284	Phosphorylation activity

Examining these domains and their likelihood to be functional in PRO-C-MG.2 reveals the following:

(1) The pkinase (protein kinase) domain (residues 230-284; page 128, lines 26-27 of the specification) was surmised from the primary sequence. Post-filing art demonstrates that this structure is well-conserved, having excellent identity with known kinases, such as GenBank NP_060241 *PX serine/threonine kinase (Homo sapiens)*. These observations strongly suggest that PRO-C-MG.2 polypeptide has kinase activity. Kinases activate and deactivate a plethora of proteins by phosphorylation and thus control the activity of those proteins (*e.g.*, signal transduction pathways, discussed at page 1, lines 21-28; as well as their use in pharmaceutical applications, page 1, lines 32-36). This protein kinase domain is also identified by many other algorithms, including BLAST analysis. ScanProsite (Gattiker et al., 2002) also confirms this finding, and as noted by Gattiker *et al.*, if an analyzed protein ". . . includes two protein kinase signatures, the probability of it being a protein kinase is close to 100%."

(2) PX kinase domain (residues 16-122; page 128, line 26); PX domains bind to phosphoinositides and occur in a variety of proteins involved in signaling pathways (Ellson et al., 2002 and references cited therein; Sato et al., 2001 and references cited therein; Xu et al., 2001 and references cited therein). PX domains are approximately 100 to 140 residues and fold into three-stranded β-sheets. The PX domain of PRO-C-MG.2 is 107 residues long.

(3) cAMP/cGMP dependent protein kinase phosphorylation sites (page 128, lines 21-22) are also a hallmark of some transcription factors, such as those that bind cAMP responsive element (CRE (Sassone-Corsi, 1995)). These kinases generally phosphorylate serine or threonine residues found close to a least two consecutive N-terminal basic residues, although there are many known sites that transgress this rule (Sassone-Corsi, 1995). However, at positions 54-58 of PRO-C-MG.2, two arginines are found N-terminally from the target serine, strongly suggesting that this site is indeed phosphorylated.

(4) PRO-C-MG.2 polypeptide has 10 putative target phosphorylation sites for the constitutively active casein kinase II (CKII). While these sites conform to two basic rules of *bona fide* CKII sites--the phosphorylated residue (*n*) being serine or threonine and having

aspartate or glutamate at the *n*+3 position (the most crucial site aside from the serine/threonine for phosphorylation) (Meggio and Pinna, 2003 and the many references cited therein)--eight of the putative sites in PRO-C-MG.2 are unlikely to be phosphorylated because they do not conform to other known characteristics of such sites. These include a predominance of aspartate and glutamate at the *n*-4 to *n*+3 positions, and the relative lack of basic residues at the crucial *n*+1 to *n*+3 sites (Meggio and Pinna, 2003 and references cited therein). The two most likely targets, **S*DLD**F (residues 190-194; full site: EKYL**S*DLD**; * indicates the phosphorylated residue) and **S*EEE**R (residues 452-456; full site: SHHG**S*EEE**), are more likely than the other eight sites to be phosphorylated, despite the basic lysine at *n*-3 in EKYLS*DLD and the two basic histidines at *n*-3 and *n*-2 in SHHGS*EEE. Similar targets with a lysine in the *n*-3 position, as well as a histidine in the *n*-3 or *n*-2 positions are known (*e.g.*, Calnexin, c-Myb, FAF-1, Hsp90α, Hsp90β, La, MDM-2, MPR300, P0, P1, P2, PHAS-1, Sp1, and TRHR proteins; *see* Meggio and Pinna, 2003 and references therein).

(5) Leucine zippers can facilitate protein binding of nucleic acids as well as protein-protein interactions (St. C. Buchanan and Gay, 1996). Although the leucine zipper exhibits characteristics that are classic to the motif, it is unlikely to behave as a nucleic acid-binding domain. Leucine zippers consist of 3 heptad (designated **a b c d e f g**) repeats which form two α-helical coils (Sassone-Corsi, 1995; St. C. Buchanan and Gay, 1996). Leucine zippers that bind DNA and polypeptides are usually found in the N-terminus and may act as transcription factors, such as the cAMP responsive transcription factors (Sassone-Corsi, 1995). However, the leucine zipper in PRO-MC-MG.2 is in the middle of the polypeptide (page 128, lines 25-26), rendering unlikely the prospect that this zipper binds DNA.

(6) The N-myristoylation sites (p. 128, line 25) are most likely non-functional for plasma membrane anchoring because they are not at the N-terminus of PRO-C-MG.2. This observation allows for the ruling out of PRO-C-MG.2 from being anchored into a membrane, although does not dismiss the possibility of the protein being transmembrane (an unlikely situation because PRO-C-MG.2 has no putative transmembrane domains).

Further sequence analysis, using algorithms available at the time of filing, also shows the following:

(1) Nuclear localization. Using PSORT II (Horton and Nakai, 1997), an algorithm that predicts various structural and cellular localizations of polypeptides, identifies two

overlapping nuclear localization motifs: RKKR, starting at residue 456; and KKRK, starting at residue 457. PSORT also identifies the leucine zipper motif, but does not identify the motif as binding DNA or otherwise being active. Finally, the algorithm predicts nuclear localization; for such proteins, 73.9% are nuclear, while the remaining are evenly divided among cytoplasmic, cytoskeletal and mitochondrial sites. Two other more recent predictive programs, SubLoc v1.0 (Hua and Sun, 2001) and Protcomp (Softberry) also predict nuclear localization.

(2) PRO-C-MG.2 possesses coiled-coil regions. Coiled-coil regions generally interact with other coiled-coil regions (Burkhard et al., 2001 and references cited therein). This motif is found in polymeric proteins, such as those of the extracellular matrix and cytoskeleton; in all of the known cytoskeletal motor proteins (myosins, kinesins and dyneins); in protein "switches" (those proteins that undergo a large conformational change in response to some event); and in molecular recognition events, such as vesicular fusion in membrane transport and binding to DNA (Burkhard et al., 2001 and references cited therein). Coiled-coils consist of two to five amphipathic α-helices that are intertwined, forming a supercoil (Burkhard et al., 2001 and references cited therein). The motif for parallel left-handed coiled-coils is characterized by heptad repeats, with apolar residues occurring in the first and fourth positions. For right-handed coiled-coils, undecad repeats are characteristic. The classic Lupas algorithm, COIL ((Lupas, 1996b; Lupas et al., 1991); based on (Parry, 1982)) and PAIRCOIL (Berger et al., 1995) both predict coiled-coil regions from approximately residue 413 to 483, with a possible kink at residues 444 to 452. Suggested controls in the Lupas routine--running the same sequence against two separate coiled-coil databases (Lupas, 1996a; Lupas et al., 1991)--confirm the results , increasing the certainty that the coiled-coil region is active. Thus, PRO-C-MG.2 is likely to physically interact with another protein.

Notable is the lack of a signal sequence in PRO-C-MG.2. Signal sequences comprise the N-terminal part of the amino acid chain and are cleaved while the protein is translocated through the endoplasmic reticulum membrane. A signal sequence would expose the polypeptide to glycosylation and dedicate the polypeptide to the secretory pathway. This analysis was done using the SignalP algorithm (Nielsen et al., 1997a; Nielsen et al., 1997b).

Because those of skill in the art would identify those domains as illustrated above and would most likely conclude that PRO-C-MG.2 is a nuclear kinase that promotes vascularization, they would know how to make and use the invention. Withdrawal of this ground of rejection is respectfully requested.

Transcriptional up-regulation rejection

In the same rejection, the Office re-asserts that the increase in transcription of *PRO-C-MG.2* does not correlate with concomitant translation, but provides no evidence that this is the case for this particular gene. Because *PRO-C-MG.2* encodes a full-length polypeptide, its expression is modulated during angiogenesis, and transcriptional control is by far the most common mechanism to determine gene expression, PRO-C-MG.2 polypeptide is almost certain to be produced at elevated levels during angiogenesis.

The overwhelming majority of gene transcripts *are* translated (Alberts, 2002); a logical result, especially in an evolutionary context. Alberts *et al.* remark that regulation at the translational level--as implied by the Office--is an exception to the rule that increases in gene expression correlate with increases in translation (Alberts *et al.* 2002 at p. 435; see also p. 379, 2nd paragraph). The Office provides no proof of the assertion that *PRO-C-MG.2* increased transcription does not result in increased translation.

To maintain its rejection, "it is encumbent upon the Patent Office [. . .]to explain why it doubts the truth or accuracy of any statement in a supporting disclosure *and to back up* assertions of its own with *acceptable evidence or reasoning*. . . " (*Marzocchi,* at 224; emphasis added). The Office has not met its burden to maintain the rejection. The rejection is respectfully requested to be withdrawn.

Claim Rejections 35 USC §112, first paragraph

The rejections of claims 24-35, 60-63, 66-67 under 35 USC § 112, first paragraph, are respectfully traversed. The specification, along with principles known in the art at the time of filing, teaches one of skill in the art how to make polypeptide variants of PRO-C-MG.2. These principles and the teachings of the specification, combined with structure-predicting algorithms, guide one of skill in the art in making functional variant PRO-C-MG.2 polypeptides.

For example, Bowie *et al.* (1990), often cited by the Office, note that while the problem of predicting protein structure from primary sequence, as well as function, can be complex ((Bowie et al., 1990); page 1306, column 1), they also note that certain general principles have been established. These principles can be applied to PRO-C-MG.2-like polypeptide variants,

which are consistent with the teachings of the specification. These principles fall in the following categories:

(1) The nature of surface *vs.* buried residues in the folded protein;

(2) The hydrophobic nature of core sequences;

(3) The interchangeable nature of surface sites; and

(4) The roles of variant residues in related sequences.

Residues that are buried in the protein require non-polar side chains (Bowie et al., 1990); while surface residue side chains are more interchangeable since few features of side chains are conserved (Bowie et al., 1990). This principle is elegantly illustrated by analysis of the λ repressor, residues that are highly conserved are buried (5 of 6), while those sites that can tolerate many different substitutions are found on the surface.

Because of their importance in folding and stability--which are driven by the hydrophobic effect--core sequences require almost exclusively hydrophobic and neutral residues (Bowie et al., 1990). While core sequences are limited to these classes of amino acids, they are mostly interchangeable with each other because the hydrophobic effect does not depend on residue pairing (Bowie et al., 1990). Even within the core, the factors of hydrophobicity, packing volume and steric compatibility are not equally "informative" (Bowie et al., 1990). While physically, these factors are all important, the factor of hydrophobicity of a sequence, rather than the factor of total side chain volume, predicts more about the side chain's acceptability as a member of the core, while the factor of steric compatibility falls midway between the two (Bowie et al., 1990).

Each surface site can accommodate many side-chain substitutions, although most proteins can tolerate only a limited number of hydrophobic substitutions overall (p. 1308, column 1, first full paragraph). This principle is due to the assumption that large patches of hydrophobic surface residues would lead to aggregation (Bowie et al., 1990), which would presumably inhibit function.

Not only would one of skill in the art recognize that Applicants were in possession of the instant invention at the time of filing, a description as filed is presumed to be adequate, unless or until sufficient evidence or reasoning to the contrary has been presented (MPEP § 2163.04; *Marzocchi*, at 224). Compliance with the written description requirement does not require an applicant to describe exactly the subject matter claimed; rather, the description must allow one of ordinary skill in the art to recognize that the applicant has invented what is claimed (MPEP §

2163.02; *In re Gosteli*, 872 F.2d 1008 at 1012; *Vas-Cath Inc. v. Mahurkar*, 935 F.2d 1555). A written description of an invention involving a chemical genus requires a precise definition, such as by structure, formula ... of the claimed subject matter sufficient to distinguish it from other materials (*Univ. of California v. Eli Lilly and Co.*, 119 F.3d 1559). Because one skilled in the art can distinguish such a formula from others and can identify many of the species that the claims encompass, such a formula is normally an adequate description of the claimed invention (*Univ. Cal.* at 1406.

The Applicants have provided such a formula. Firstly, the sequence of PRO-C-MG.2 is given in SEQ ID NO:2. Furthermore, an art-accepted method for calculating sequence identity has also been provided (page 16, line 5 to page 19, line 18). The specification specifically teaches variants of SEQ ID NO:2; for example, see line page 35, line 18 to page 36 line 19 (chimeric and fusion polypeptides comprising the polypeptide sequence of SEQ ID NO:2) and page 31, line 36 to page 32, line 15 for variants wherein conservative amino acid substitutions have been made.

The instant specification would convey with clarity to those skilled in the art that, as of the filing date, Applicants were in possession of the claimed subject matter. The law, as articulated by the Federal Circuit, requires no more (*Vas-Cath* at 1563). Possession is also demonstrated by the analysis, *supra*, regarding the details of the polypeptide of SEQ ID NO:2.

Applicants submit that the written description requirement has been met. Withdrawal of this ground of rejection is respectfully requested.

Objections

The objections to claims 26 and 63 have been obviated by amendment.

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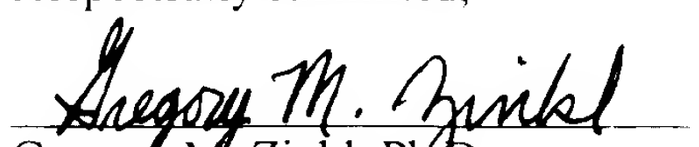
Reconsideration and withdrawal of all claim rejections are respectfully requested. Applicants believe that all claims in the present application are in condition for allowance.

Should the Examiner have any questions, or would like to discuss any matters in connection with the present application, the Examiner is invited to contact the undersigned at (312) 876-8936.

Respectfully submitted,



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